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The Etiology of Diphtheria,

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366





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THE ETIOLOGY OF DIPHTHERIA.¹

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CONCERNING the origin of diphtheria much discussion has arisen. Although its infective character has been doubted by some, it is now quite universally accepted; and I shall confine my remarks to a brief review and discussion of the etiology of the disease from the standpoint of the biologist.

I shall adhere to the classification of micro-organisms that is now universally adopted, viz., using the term "bacteria" in a generic sense, including both the

Micrococci, the ball-forms, and the
Bacilli, the rod-forms.

That bacteria were early found in diphtheritic membrane, even before the recent improvement and perfection of the microscope permitted the researches and investigations resulting in the discovery of many pathogenic micro-organisms, is not to be wondered at, when we realize that the healthy human mouth is constantly infested with bacteria of various kinds; not less than thirty different varieties having been isolated and cultivated by my friend Prof. W. D. Miller, whose labors have won for him the distinction of being the only American who has been honored with a professorship in the University of Berlin. We must remember, however, that it is one thing to prove the existence of micro-

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organisms in a diphtheritic false membrane, and another thing to prove that these germs are the *cause of the disease*. The difficulties are very great, and in the case of diphtheria as perhaps in no other disease, do we realize the importance of the isolation of the bacteria and their cultivation in a pure state, together with the reproduction of the disease by inoculation of the cultivated germs, before an attempt can be made to judge whether they are present as the cause of the disease, or are there, as most of them undoubtedly are, only as a result of the diseased state affording favorable conditions and soil for their growth and development.

The first reference to the idea that diphtheria is of parasitic origin, that I have found, is an article by Prof. Laccock,² and the idea was afterwards revived by Jodin.³

Oertel⁴ says concerning bacteria in diphtheria: "They were discovered as far back as 1868 by Buhl, Hueter, and myself [I called them at that time micrococcus] in false membranes, the blood, and the tissues; in like manner they were demonstrated by von Recklinghausen, Nassiloff, Waldeyer, Klebs, Eberth, Heiberg and others, in the most different organs and tissues. In secondary infection of wounds, tracheotomy incisions, and ulcers, the grayish skin-like false membranes, as well as the tissues themselves, are crowded with these organisms."

In a "Treatise on Diphtheria," 1880, Dr. A. Jacobi reminds us that "Buhl was the first to discover schizomycetæ in diphtheritic membrane, but expressed no opinion as to the part they played in the process." Hüter found them in the gray diphtheritic covering of wounds, in the surrounding appar-

² Medical Times and Gazette, May 29, 1858.

³ "De la nature et du traitement du croup, etc.," *Revue Méd.*, t. 1. pp. 22 and 134, Paris, 1859.

⁴ *Cyclopædia of the Practice of Medicine*, Ziemssen, American edition, vol. 1. page 528.

ently healthy tissues, and in the blood. Hüter and Tomasi found them in the diphtheritic membranes of the pharynx and larynx, inoculated them on the mucous membranes of animals, and described them as small, round or oval, dark-colored, active little bodies. The latter observers look upon these organisms as a part of the infectious element. Oertel found them in diphtheritic membrane and in inflamed mucous membranes, in the lymphatic vessels, lymphatic glands, kidneys and other organs; he considers them at the bottom of the diphtheritic process and constituting the contagious element. Nassiloff, too, after inoculation in the cornea, noticed an enormous multiplication of the microscopic organisms, and their appearance with pus-cells in the lacteals, and in the lymphatics of the palate, and even in the bones and cartilages. He asserts that the development of organisms is the primary step in the diphtheritic process. Eberth made successful inoculations in living tissues: the microorganisms, introduced into the cornea, proliferated actively and caused an inflammation of irritative character in the surrounding tissue. He asserts⁵ that diphtheria cannot occur without bacteria. Klebs inoculated the micrococci in pigeons and dogs and demonstrated the presence thereof in the blood of the animals after death. Orth found them in the pleura, lungs, kidneys, and urinary bladder."

Giacchi⁶ believes that a parasite is as necessary in the pathogenesis of diphtheria as the *Oidium vitis* is in the production of the disease of the grape.

Letzerich⁷ also differs from other German observers in regarding a true fungus, *Zygodermus fuscus*, as the specific contagion of diphtheria.

⁵ Zur Kenntn. der bacterit. Mykosm, 1872.

⁶ "Natura e therapia dell'angina ditteria," Lo Sperimentale, Nov., 1872.

⁷ Virchow's Archiv, Bd. xlv. et seq.

The *Micrococcus diphtheriæ*, Oertel,⁸ is thus described: "It has an oval form, with a length of 1 to 1.5μ and a breadth of 0.3μ ; larger individuals, found nearer the surface, being 4.2μ long, and 1.1μ broad. Where the individuals are more scattered, they occur mostly in pairs, rarely a number connected into a torula-like chain. When present in masses, the cells lie so close together that it is difficult to determine whether they are connected or not. They are then imbedded in a gelatinous envelope, and thus combined in masses into a colony."

Talamon⁹ does not recognize the *Zygodesmus fuscus* of Letzerich nor the *Microsporon* of Klebs as the cause of diphtheria, which he believes to be a mycelium with characteristic growths from 2 to 4 or 5μ size, and having two kinds of spores:—

a. round or oval spores, which are the spores of germination, which occur in zooglea, and

b. rectangular spores, which represent the third term of development of the fungus. These he has cultivated and inoculated on the mucous membrane of the mouth and nose of six rabbits, two guinea pigs, four frogs, one cock, and four pigeons, with reproduction of the membrane and death of some of the animals.

Klebs¹⁰ mentions that at first he supposed there was only one form of micro-organism present in diphtheria. This he called the *Microsporon diphtheriticum*, and he claimed that it produced both rods and cocci, as different forms in the development of the same organism. Afterwards, however, he says that he recognized another form of diphtheria, which was characterized by the presence of bacilli

⁸ Zur Ätiologie der Infektionskrankheiten, 1881; and Journal Roy. Mic. Soc., ser. II. vol. II. p. 88.

⁹ Progrès Méd., 1881, ix. pp. 122 and 49.

¹⁰ Verhandlungen des Congresses fuer innere Medicin, 1883, pp. 139 to 154.

The later form he found at Zurich. It corresponded with the first form only in the gross anatomical changes. The latter form is characterized by the tendency to an extremely rapid extension of the membrane into the trachea, even while the affection in the pharynx is still in active process. Death usually occurs from suffocation.

Morphologically, Klebs says that the bacilli are long and narrow and that they hardly attain the size of the bacillus tuberculosis. Two spores are always found in each rod. When the diphtheritic membrane is dried gradually over sulphuric acid at the ordinary temperature, the spores increase very rapidly, and then rods may be found which contain no spores, while others contain four spores. He is convinced, he says, that a true diphtheria exists only when rod-shaped organisms are present in the membrane. This allows of two possibilities: in the microspore form we have micrococci, together with somewhat long rods which do not contain spores, and in these cases a general infection is rapidly developed. In the bacillus form, on the contrary, which is first dangerous on account of its rapid extension on the mucous membranes, we find a great number of small rods which contain from two to four spores.

We learn from the address of Dr. E. G. Barnes¹¹ that Loeffler, whose investigations were extensive and are published by the New Sydenham Society, found, in the cases he examined, two organisms present in large numbers; the one were chain-forming micrococci or streptococci; the other the bacilli described by Klebs as characteristic of diphtheria. The streptococci may be exonerated from being the active cause of diphtheria by the fact that they are present in various other diseases which are accom-

¹¹ British Medical Journal, July 28, 1888.

panied by lesions of the mucous membrane, for example, small-pox, typhoid and puerperal fever, and therefore may be regarded as accidental; that they are found only in a limited number of cases of human diphtheria, and that, when inoculated on lower animals, they never produced a disease even resembling it. Much stronger evidence was shown by Loeffler in favor of Klebs' bacillus being the true cause, and he even produced a similar disease by inoculating them on lower animals; but, on the other hand, he found they were not present in a number of undoubted cases of diphtheria; that in the false membrane he produced by introducing them through a wound in the trachea in rabbits and fowls, he did not find them in the same typical arrangement as in man: that they produced no effect in several animals otherwise susceptible to their action when applied to the uninjured mucous membrane of the fauces, respiratory passages, eyes and vagina; that paralytic symptoms did not occur in the inoculated animals; and, lastly, that in one case he found a perfectly indistinguishable bacterium in the saliva of a healthy child.

Many allusions are now being made in the secular press to the work recently done in Pasteur's laboratory, which has been described in the *Gazette Hebdomadaire de Med. et de Chir.*, January 18, 1889. MM. Roux and Yersin constantly found the bacillus of Klebs and Loeffler, which they describe to be a little thicker than the bacillus tuberculosis and of the same length. This description, you will observe, differs a little from the original description of Klebs quoted above. They have cultivated the bacillus; and their inoculation experiments have produced paralysis, without which they do not consider the proof of real diphtheria conclusive.

My own experiments in the cultivation and in-

oculation of the bacteria of diphtheria were made several years ago, and are reported in a paper read before the meeting of the Eighth International Medical Congress at Copenhagen in 1884.¹²

In November, 1883, I assisted Dr. H. O. Marcy in performing the operation of tracheotomy upon a child three years old, who was suffering from a severe attack of diphtheria. He was *in extremis* at the time of the operation, the breathing being very short and difficult. Membrane covered both tonsils. The operation was successfully performed and a tube inserted, when the breathing became perfectly free. Previous to the introduction of the tube, a complete membranous cast of the trachea was removed through the opening. The subsequent history was unfavorable; for the child died of blood poisoning about thirty-six hours later.

Soon after the operation I inoculated one of my culture-bulbs with a small piece of the membrane removed from the trachea. These bulbs are made after those of Sternberg of the United States army, which I then preferred to the method of culture on solid culture-media; I had not become thoroughly familiar with the latter method until some months later in Berlin, when I was soon convinced that it affords many advantages that cannot be obtained from cultures in bulbs. These bulbs are made from ordinary glass-tubing about three-tenths of an inch in diameter. In one end, a bulb is blown and the other extremity is drawn to a fine capillary point. These I made myself in quite large quantities at a time. They were filled two-thirds full with a sterilized beef-bouillon, then hermetically sealed, and in this condition they will keep indefinitely if successfully made.

¹² Compte-rendu de Congrès Périodique International des Sciences Médicales, 8me session, Copenhagen, 1884, t. i. Section de Pathologie Général et d'Anatomie Pathologique, p. 114.

For cultures I found it best to use bulbs which had stood the test of a temperature of 70° to 100° F. for several weeks; for if they remain clear and pellucid at the end of this time, any subsequent changes that might occur are due to the substances introduced.

Four days after the introduction of the diphtheritic membrane as seed, the liquid in the culture-bulb kept at temperature of 70° became cloudy or turbid; and when examined with the microscope at 1000 diameters, there were found immense quantities of a micrococcus, identical with those seen in the fresh membrane. This micrococcus has about the diameter of the micrococcus of pus, and is very slightly elongated. They were grouped in clusters of a few members each, and belong to the group of staphylococci.

A second culture-bulb was inoculated with a fraction of a drop of the liquid in the first, and three days later the same cloudy appearance was noticed, and examination showed identical micrococci. In this way the cultures were carried through ten generations, in each case several bulbs being inoculated at a time, and each one breeding true in three days. In all, about fifty bulbs were used.

My subsequent experiments of inoculation were carried on with the advice and assistance of Dr. Wm. F. Whitney. Four guinea-pigs were inoculated in the cornea with the contents of one of the bulbs containing the culture of the sixth generation. One of these animals died thirty-six hours later of blood poisoning. The others became very ill, losing their appetite and the eyelids becoming much swollen and oedematous, with profuse discharge which contained the micrococci. The cornea became cloudy and was covered with a membrane. Two of these animals were killed on the third day after inocula-

tion, this being the period at which the micrococci developed : one was allowed to get well, but the eye was completely destroyed.

In the aqueous humor of the eyes dissected there were found micrococci, which were also found in sections of the cornea of the eyes, which had been placed in alcohol immediately after removal, and when hardened were cut with a microtome.

These experiments are limited in number and, I know, need further confirmation ; but as far as they go they seem to show that there is a micrococcus of diphtheria which can be cultivated, and which when inoculated in the guinea-pig produces diphtheria.

If, however, further proof is needed I can give it ; for on the third day after killing the animals, and after no other exposure, I myself became ill, developing a severe attack of diphtheria, which appeared first in one tonsil and the uvula, and then on the other tonsil ; being accompanied with severe constitutional symptoms and followed by a slow and tedious recovery. This has proved, to my satisfaction at least, the correctness of these views.

Thus we see in brief review the chain of the sequence of events : —

A typical case of diphtheria in a child ;

The presence of micrococci in the membrane ;

The cultivation of the micrococci in pure cultures to the tenth generation ;

The inoculation of guinea pigs with micrococci of the sixth cultivated generation, and reproduction of the disease ;

The unwitting inoculation of the experimenter, thus bringing the disease back to its original form in a human being.

